The Fetal Basis of Adult Disease: Role of the Environment

It is recognized that 2–5% of all live-born infants have a major developmental defect. Approximately 40% of these defects are thought to be due to the effect(s) of an adverse exposure of a genetically predisposed fetus to intrauterine environmental factors. It is now clear that in many cases the fetus is more sensitive than the adult to the same environmental insults. Exposure to environmental agents during early development can result in death, structural malformation, and/or functional alteration of the embryo or fetus. These toxicant-induced pathogenic responses are most likely the result of altered gene expression associated with altered cell production and cell differentiation involved in the establishment of cell lineages leading to the structural and functional character of the tissues, organs, and systems that arise from these lineages.

The NIEHS has a significant program that addresses the role of developmental exposures on structural malformations and on functional alterations whose effects are readily observable early in development. The purpose of this program announcement (PA) with a set-aside of funds and a Special Emphasis Panel review by the NIH Center for Scientific Review is to stimulate research in an important and emerging area of developmental toxicology: the effects of *in utero* exposures that cause permanent functional changes that are not overtly, grossly teratogenic, yet that result in increased susceptibility to disease/dysfunction later in the life span. This PA seeks to encourage the application of new high-throughput functional genomic, metabonomic, proteomic, and bioinformatic technologies to pursue an understanding of these latent effects of *in utero* environmental insult.

The underlying scientific hypothesis behind the fetal basis of adult disease has been developed by epidemiologic studies and emphasized by Dr. David Barker in the United Kingdom. Most of the supporting studies in this area have concentrated on grossly altered nutrition *in utero* and its striking influence on multiple aspects of adult health and disease risk. Dr. Barker has shown that during development, fetuses respond to severe malnutrition by favoring the metabolic demands of the growing brain/central nervous system and heart at the expense of other tissues. The growing brain/central nervous system and heart tissue may not, however, escape entirely unscathed. The long-term consequences of this response are that the fetus is live-born but is more prone to diseases later in life.

In support of the Barker hypothesis, epidemiologic studies have shown that markers of malnutrition, such as low birth weight, small for gestational age, or frank intrauterine growth retardation (IUGR) strongly predict the subsequent occurrence of hypertension, hyperlipidemia, insulin resistance, type 2 diabetes mellitus, ischemic heart disease, breast cancer, and prostate cancer in adult life. Fetuses that are clinically malnourished during the first trimester of development are three times more likely to be obese as adults. In addition to malnutrition, environmental exposures present during *in utero* development can have profound influences on fetal growth. Evidence has been presented in human populations that heavy exposure to PM_{10} air pollution containing carcinogenic polycyclic aromatic hydrocarbons can be correlated with increased IUGR with a peak impact in the earlier portion of the first trimester—a most vulnerable period of the cell lineage expansion, differentiation, and cell interaction events of organogenesis and first growth.

The concept of fetal programming of structural–functional formations during development has been proposed to explain these findings, and the resultant research area is referred to as fetal basis of adult disease, or FeBAD, research. "Programming" is the term used to describe lifelong changes in function that follow a particular event in an earlier period of the life span. While epidemiologic studies have identified the phenomenon of metabolic programming, little is known about the mechanism(s) by which fetal insults lead to altered programming and to disease later in life. Emphasis thus far has been on alterations in nutrition during development with virtually no focus on the role that exposures to environmental agents—either alone or in combination with qualitative alterations in macro- or micronutrition—might have on this phenomenon. There is evidence that some environmental agents, especially those with endocrine agonist or antagonist activity, may alter developmental programming via alterations in gene expression or gene imprinting that do not result in malformations but in functional deficits that do not become apparent until later in life. In the reproductive tract, the classic example of this phenomenon in the environmental area is the diethylstilbestrol (DES) story. In humans, *in utero* exposure to DES leads to an increase in vaginal adenocarcinoma around the time of puberty. In mice, neonatal DES exposure leads to an increase in uterine adenocarcinoma in adulthood. While the direct connection has not been made between *in utero* programming changes due to DES and later-life disease, it is known that DES (in the animal studies) results in altered gene expression in the uterus that is irreversible without any noticeable gross alterations in uterine morphology.

Cardiopulmonary diseases in postnatal life have also been linked to prenatal exposure. The best-known example is the association between low birth weight (which is associated with poor maternal nutrition and perhaps corticosteroid exposure) and cardiovascular disease (e.g., myocardial infarcts), predictors of future cardiovascular disease such as hypertension and atherosclerosis, and complex metabolic diseases such as diabetes. In addition, studies have shown that maternal smoking is associated with deficits in lung function and with asthma symptoms in the offspring. Data indicate that these associations are independent of smoking status after birth.

Some forms of neurodegenerative disease may have their origins in *in utero* exposures. For example, there is preliminary evidence that a bacterial stimulus (endotoxin) can produce cytokines that impair the development of the mesencephalic dopaminergic systems during pregnancy. This attenuation of the dopamine neurons during fetal development leaves the offspring with fewer dopaminergic neurons at birth and at possible increased risk for Parkinson disease in later life. In a similar vein, there is preliminary evidence that exposure to environmental neurotoxicants during dopaminergic development enhances the susceptibility to accelerated dopaminergic cell death during aging via the common molecular mechanisms of the alteration of stress-activated signal transduction pathways, expression of differentiation transcription factors, survival factors, or phenotype marker proteins in the nigral dopaminergic neurons. Similarly, there is evidence that *in utero* exposure to polycyclic biphenyls leads to altered thyroid function and subsequent learning disabilities later in life. In all instances, data are needed to show that the *in utero* exposures actually lead to an altered programming at the molecular level and that the disease/dysfunction is a direct result, albeit temporally discordant in its onset and/or progression, of that altered programming.

Another promising area for investigation is how environmental prenatal exposures might alter immune system programming. The development of the immune system, including the development of the repertoire of reactive lymphocytes that will exist in postnatal life, begins prenatally. Alterations of the fetal immune environment might preprogram the highly sensitive fetal immune system for aberrant immune regulation, leading to a loss of tolerance to self-antigens and resulting in an increased risk for autoimmune disease. These changes might manifest in adult life and perhaps only after a second exposure to related environmental chemicals. There is evidence, for instance, that mice exposed prenatally to estrogenic compounds appear to develop normal immune systems. However, when stimulated with certain environmental chemicals, they can show an increased susceptibility to autoimmune disease. Similarly, there is evidence in humans and experimental animals that prenatal exposure to immunosuppressive drugs can lead to immune alterations in the mature animals.

Based on the epidemiologic data that support the Barker hypothesis and the preliminary data showing alterations in gene expression and imprinting due to *in utero* exposures to some environmental agents, we propose that exposure to certain environmental chemicals as well as altered nutrition, or in combination with altered nutrition, will in some situations lead not to easily identifiable structural malformations, but instead to alterations in developmental programming expressed as a permanently altered gland, organ, or system potential. These states of altered potential would be a result of changes in gene expression due to altered imprinting, and the underlining methylation-related protein–DNA relationships associated with chromatin remodeling. These effects may occur in a time- and tissue-specific manner, and

such alterations may be irreversible. The end result is an animal that is sensitized such that it will be more susceptible to diseases later in life.

The pathophysiology or functional change that results from the exposures/insult could lead to 1) the occurrence of a disease that otherwise would not have happened, 2) an increase in risk for a disease that would normally be of lower prevalence, or 3) either an earlier onset of a disease that would normally have occurred or an exacerbation of the disease. Finally, the pathophysiology could have a variable latent period (depending on the toxicant, time of exposure, and tissue/organ affected) and potentially transgenerational effects.

Research approaches relevant to this PA include the following:

1) To provide a sound mechanistic understanding of fetal programming of adult disease, studies supported by this initiative must involve whole-animal developmental exposures during gestation. Applicants can propose studies using transgenics, model organisms, or rodent models. For the purpose of this initiative, human studies (clinical or epidemiologic) are not responsive.

2) Applicants must study an environmental agent/chemical/stressor to which there is human exposure and the potential for *in utero* exposure. This includes any endocrine-active chemicals or organic solvents, particulate matter, pesticides, nutritional supplements, phytochemicals, or metals. Nutrition alone cannot be used as an *in utero* exposure but can be studied in conjunction with another exposure.

3) Applications must propose studies that focus on *in utero* exposures, but additional exposures at other time points (e.g., exposure beginning *in utero* and extending to postnatal period, exposure *in utero* followed by adult exposure) can be included.

4) This initiative requires the use of the new technologies of gene expression profiling, and where appropriate, the examination of epigenetics (methylation, imprinting, chromatin remodeling).

5) Applications must link *in utero* exposures to changes in gene expression that are tissue-specific and irreversible. These changes in gene expression will then need to be measured in the adult and correlated with the disease/dysfunction studied.

6) A specific adult-onset disease/dysfunction must be the focus of the application, with emphasis on the role of *in utero* exposure and changes in gene expression in the fetus to the adult onset or severity of the disease. Applications that are not focused on a specific adult disease/dysfunction are not responsive. For example, applications that focus on *in utero* exposures as triggers of diseases of childhood or puberty are not responsive to this specific announcement.

7) Applications must focus on one of the following four emphasis areas: the reproductive tract, the pulmonocardiovascular system, the brain/central nervous system, or the immune/autoimmune system. Diseases of other tissues or organ systems are not responsive to this specific announcement. The disease categories of special interest to the NIEHS with respect to this initiative include reproductive/hormonal (fertility; endometriosis; fibroids; premature menopause; polycystic ovary syndrome; prostate, ovary, or breast cancer), cardiopulmonary (heart disease, atherosclerosis, hypertension, chronic obstructive pulmonary disease, adult asthma), brain/central nervous system (neurodegenerative diseases such as Parkinson disease, Alzheimer disease), and immune/autoimmune (altered immune responsiveness, systemic or tissue-specific autoimmine diseases of adulthood). It may be possible to submit applications to this initiative with an emphasis on other diseases as long as they are related to one or more of the above noted four emphasis areas. It should be noted that these are all adult-onset diseases.

8) Critical areas of expertise that are required of applicants include developmental biology/toxicology, disease pathophysiology, and gene expression profiling, including data analysis and interpretation of global gene expression alterations.

9) The National Cancer Institute (NCI) is interested in funding research aimed at understanding the effects of biological, chemical, and radiologic exposures *in utero* that cause permanent functional changes resulting in increased susceptibility to cancer in adult life. The specific etiologic agents of interest include microorganisms such as herpes virus, HHV8/KSHV, cytomegalo virus, papilloma virus, polyoma virus, and bacterial infections such as chlamydia, to name a few. Chemical agents that have been characterized as carcinogenic or that are suspected to be carcinogenic in humans—including polycyclic aromatic hydrocarbons, nitrosamines, heterocyclic amines, aromatic amines, DES, and estrogens, as well as metals and metalloids such as chromium, mercury, and arsenic—are of interest to the NCI. Additionally, exposures to radiologic agents from all externally applied sources as well as exposure to internally deposited radionuclides that would result in *in utero* exposures are of interest in this context. Studies that link *in utero* exposures to such agents that result in permanent alterations in gene expression in tissues of the reproductive system, the pulmonocardiovascular system, the brain/central nervous system, or the immune/autoimmune system leading to or resulting in cancer in those organ sites later in the adult life of the exposed fetus are sought by the NCI.

10) The NCI is also interested in funding research aimed at understanding the consequences of fetal exposure to toxicants, hormone agonists, or antagonists that alter the expression or function of the steroid nuclear receptor superfamily of genes (androgen, estrogen, progesterone, glucocorticoid, vitamin D_3, thyroxine) in normal or cancerous organs of the male and female reproductive tract and/or immune system. The NCI is further interested in the consequences of environmental carcinogen or tobacco smoke exposure of mice during gestation that result in long-lasting immune function deficiencies or inflammatory responses, insofar as they are related to the development of hematological malignancies or cancers of the reproductive system, the brain/central nervous system, and/or the lung. Studies that link fetal exposure to toxicants or other substances affecting somatic stem cells that later populate organs such as the mammary gland, the prostate gland, and the lung, and that have cancer as the end point, are sought by the NCI.

This PA will use the NIH exploratory/developmental (R21) award mechanism.The R21 grant award mechanism supports innovative, high-risk/high-impact research requiring preliminary testing or development; exploration of the use of approaches and concepts new to a particular substantive area; and research and development of data upon which significant future research may be built. Applications will be considered high-impact if they demonstrate the potential for groundbreaking, precedent-setting significance, and high-risk if they either lack sufficient preliminary data to ensure their feasibility or involve the use of a new model system or technique.

This PA uses just-in-time concepts. It also uses the modular budgeting format (see http://grants.nih.gov/grants/funding/modular/modular.htm). Specifically, if you are submitting an application with direct costs in each year of $250,000 or less, use the modular format. This program does not require cost sharing as defined in the current NIH Grants Policy Statement at http://grants.nih.gov/grants/policy/nihgps_2001/part_i_1.htm. It is anticipated that approximately $2 million in fiscal years 2004 and 2005 will be available to fund grants in response to this PA. An applicant for an R21 grant may request a project period of up to three years and a budget for total direct costs, including third-party facilities and administrative costs, not to exceed $100,000 per year.

The deadline for receipt of letters of intent is 10 July 2004, with 12 August 2004 the deadline for receipt of applications. Applications must be prepared using the PHS 398 research grant application instructions and forms (rev. 5/2001). The PHS 398 is available at http://grants.nih.gov/grants/funding/phs398/phs398.html in an interactive format. Complete information on this PA is located online at http://grants.nih.gov/grants/guide/pa-files/PAR-03-121.html.

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